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Synthesis of some modified 2′–5′-linked oligoriboadenylates of 2–5A core

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Abstract. The synthesis of three analogues of 2′–5′-linked oligoriboadenylates is reported. Two trimers, having at the 2′-end a 3′-amino- (or 3′-tetradecanamido)-3′-deoxy-riboadenosine, were assembled via a phosphotriester approach. In the same way, we also prepared a tetramer carrying at the 2′-end β-L-riboadenosine instead of β-D-riboadenosine. The non-terminal building unit 3′,5′-*O*--(tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine and the appropriately protected modified riboadenosines were coupled using the phosphorylating agent 2-chlorophenyl-*O,O-bis*(1-benzotriazolyl) phosphate. The removal of all protecting groups from the intermediate trimers and tetramer was effected by ammonolysis, followed by treatment with fluoride ions.

Introduction

Interferon-treated cells and lysates of rabbit reticulocytes contain *inter alia* the enzyme 2–5A synthetase which produces[1] 2′–5′-oligoadenylic acids (2–5A)** from ATP in the presence of double-stranded RNA. It has been proposed that 2–5A plays an important role in the mechanism of antiviral action of interferon[2]. According to this mechanism, 2–5A, or its core, activates a latent 2′,5′-oligoadenylate-dependent endonuclease (RNase L) in mammalian cells. Binding of these specifically linked oligomers to RNase L results in the hydrolysis of messenger RNA and consequently the inhibition of protein synthesis. The life-time, however, of naturally occurring 2′,2′-oligoadenylates in cell-free systems is severely restricted by the presence of an exonucleolytic 2′,5′--phosphodiesterase[3] (2′-PDi) which rapidly hydrolyses the 2′–5′-phosphodiester linkages of 2–5A. With the objective of decreasing, or suppressing completely, the hydrolytic action of 2′-PDi, many analogues of 2–5A, and of core 2–5A, have been synthesized[4] in the last few years. Most of these 2–5A analogues were primarily tailored to survive the exonucleolytic action of 2′-PDi. They failed, however, to mimic adequately the activation process of RNase L[5].

Thus far, only analogues of 2–5A, in which the ribose of the 2′-terminal ribonucleotide was transformed into an *N*-substi-

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** Abbreviations: 2–5A, general formula ppp5′A2′(p5′A)$_n$, refers to 2′–5′-linked oligoriboadenylates ($n = 2$ is minimum number for optimal biological activity) bearing at the 5′-end a triphosphate group. Core 2–5A, general formula A2′(p5′A)$_n$ ($n = 2$ is minimum number for optimal biological activity), lacks the triphosphate function.

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tuted morpholine[6] (azahexapyranose), proved to be resistant to 2'-PDi and potent activators of RNase L. The latter results seem to indicate that chemical modifications of 2–5A are only allowed in the terminal ribofuranosyl unit of the 2'--riboadenosine moiety of the molecule. We here report the synthesis of three core analogues of 2–5A, trimers **16a,b** [$n = 2$, $R^1 = H$ or $C(O)CH_2(CH_2)_{11}CH_3$] and tetramer **17** ($n = 3$), where, in each case, a differently modified ribofuranosyl moiety replaces the β-D-ribofuranosyl of the 2'-terminal nucleotide of the 2–5A core structure.

Results and discussion

Our first objective was the synthesis of two 2'–5'-linked trimers (*i.e.* **16a,b**) containing at the 2'-end riboadenosyl moieties in which the 3'-hydroxyl groups are replaced by an acylated (**16a**) or free-amino function (**16b**), respectively. The preparation of the properly protected and *N*-acylated 2'-terminal residue **5a** is outlined in Scheme 1. Condensation of **1**[7] with persilylated N^6-benzoyladenine[8] (**2**), in the presence of trimethylsilyl trifluoromethanesulfonate[9], yielded the β-D--ribofuranosyladenosine **3** in a reasonable yield. Short treatment of **3** with sodium methoxide afforded **4** which, after hydrogenation of the azido function in the presence of

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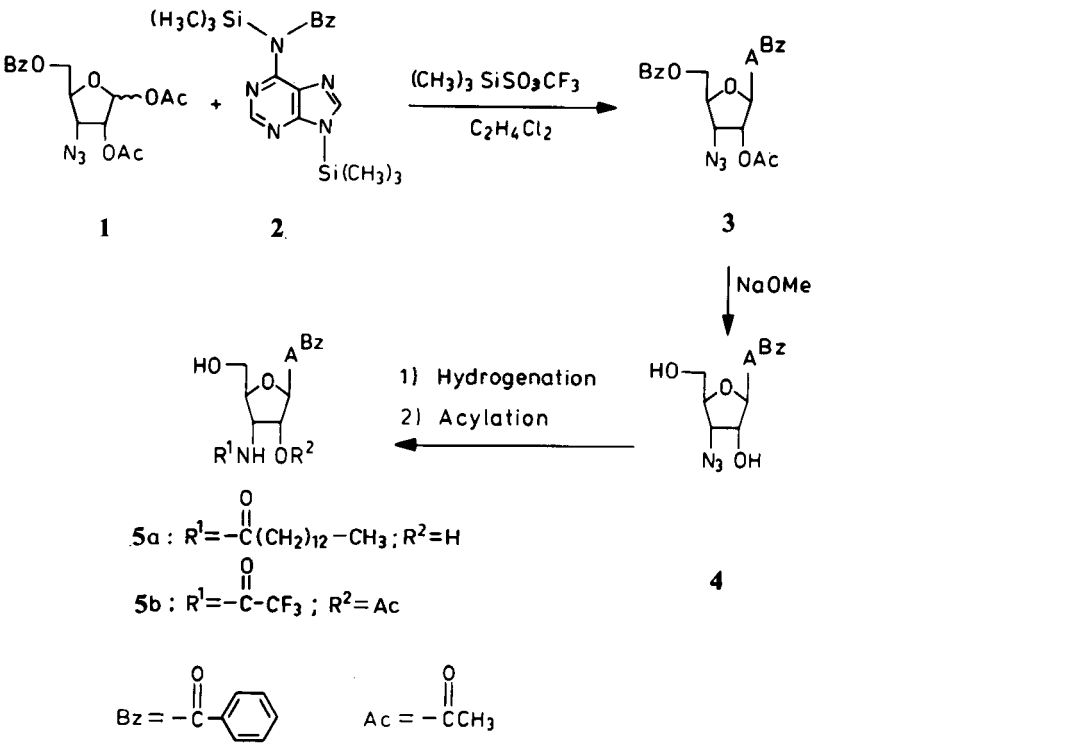
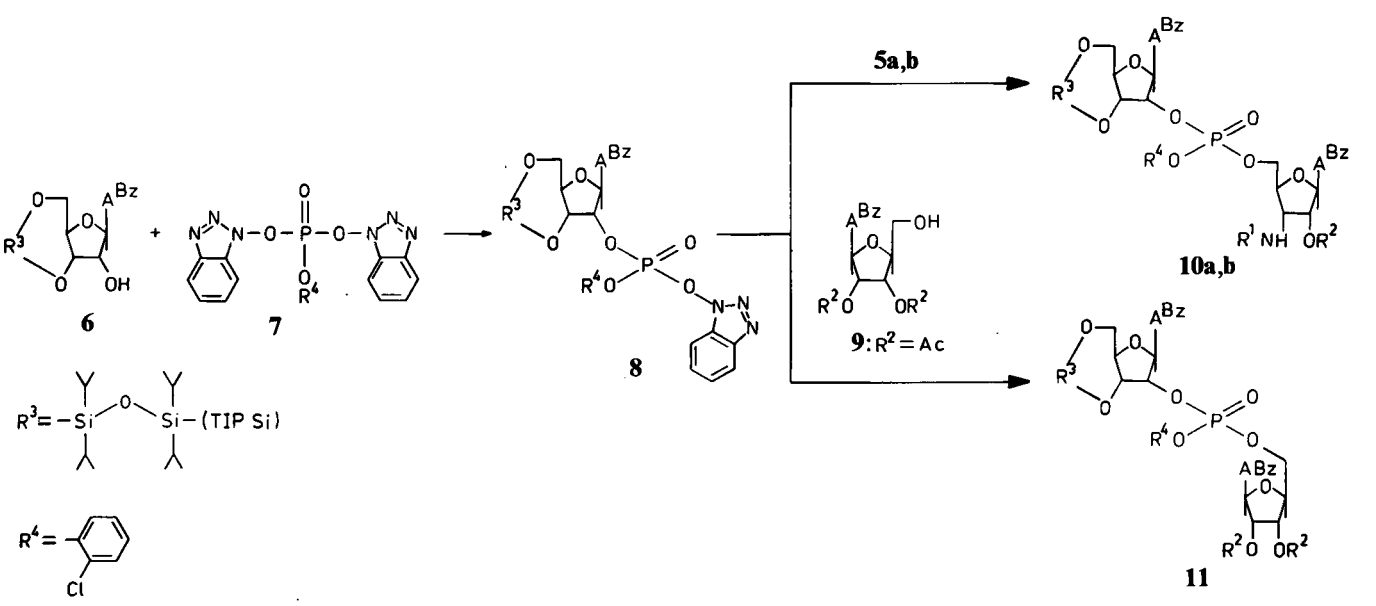


Scheme 1



Scheme 2

palladium on charcoal, followed by acylation of the free-amino function thus obtained with tetradecanoyl chloride, gave terminal unit **5a** having the 2'- and 5'-OH groups free. The other terminal unit **5b**, carrying a free 5'-OH group, was prepared as previously reported[10].

The formation of the intermediate 2'–5'-phosphotriester linkages between units **5a,b** and the non-terminal unit **6** to afford the dimers **10a,b**, respectively, is illustrated in Scheme 2. A solution of crystalline 3',5'-*O*-(tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine (**6**)[11] in pyridine was treated with a slight excess of the bifunctional phosphorylating agent **7**[12] to give the intermediate phosphotriester **8**. Condensation of **8** with **5a**, in the presence of *N*-methylimidazole, afforded, after work-up and purification by short-column chromatography[13], homogeneous dimer **10a**. In the same way, dimer **10b** was obtained by the coupling of **5b** with **8**. Yields and other relevant data on the synthesis of dimers **10a,b** are recorded in Table I. Selective ring opening (see Scheme 3) of the 3',5'-*O*-tetraisopropyldisiloxane function in dimer **10a** with diluted hydrochloric acid[14,4c] resulted in the formation of **12a** having a free 5'-OH. The latter was now extended by the addition of **8** to give trimer **14a**. In the same way, **10b** was elongated to afford trimer **14b**. Yields and other analytical data on the synthesis of dimers **12a,b** and trimers **14a,b** are recorded in Tables II and III, respectively.

Complete deblocking of trimers **14a,b** was effected by ammonolysis [removal of the 2-chlorophenyl (R^4), benzoyl (Bz), trifluoroacetyl (R^1 from **14b**) and acetyl (R^2 from **14b**)] followed by treatment with tetrabutylammonium fluoride (removal of silyl groups R^5 and R^3). Crude trimers thus obtained were purified by anion-exchange chromatography and further processed (see Experimental) to give, as judged from ^{1}H NMR spectroscopy, the sodium salts of **16a,b** as homogeneous solids. The synthesis of the 2'–5'-linked tetramer **17** (n = 3), having at the 2'-terminal a β-L-riboadenosine instead of the naturally occurring β-D-enantiomer, was accomplished as described for the preparation of trimers **16a,b**. Thus, coupling of the appropriately protected L-isomer **9**[15] with **8** (see Scheme 2) yields the fully protected dimer **11** which, after acidolysis and subsequently addition of **8** (see Scheme 3), was converted into trimer **15** (n = 1). The fully protected tetramer **15** (n = 2) was obtained by performing on **15** (n = 1) the same two-step procedure as applied to the extension of dimer **11** to give trimer **15** (n = 1). Yields and other relevant data on the synthesis of tetramer **15** (n = 2) are summarized in Table III. Complete removal of all protecting

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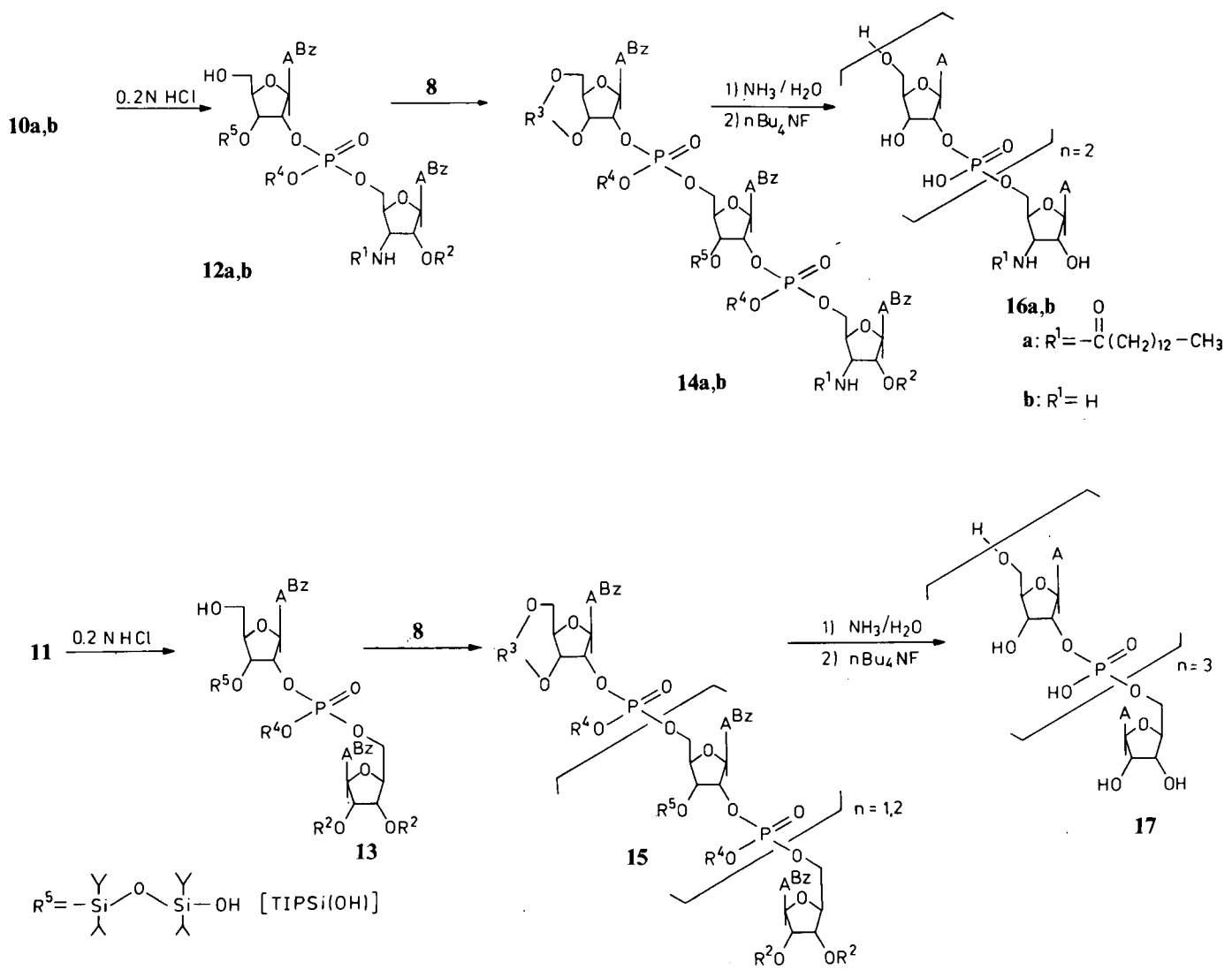

Scheme 3

Table I Relevant data on the synthesis of **10a,b** *and* **11**

Starting compounds	Quantity (mmole)	Base (ml)	Reaction time (min)	Products yield (%)	^{31}P NMR[a] data	R_f values[b]
1st component **8** 2nd component **5a**	1.0 0.8	*N*-methyl-imidazole (0.4)	90	**10a** (77.5)	−6.95 −8.05	0.62 0.60
1st component **8** 2nd component **5b**	0.68 0.68	Pyridine (0.4)	150	**10b** (56.3)	−7.35 −8.11	0.75 0.73
1st component **8** 2nd component **9**	0.75 0.65	*N*-methyl-imidazole (0.3)	90	**11** (88)	−7.38 −7.45	0.68 0.66

[a] δ Values are given in ppm; external reference H_3PO_4; solvent $CDCl_3$. [b] Mixture of diastereoisomers (System B).

Table II Relevant data on the products obtained after acidolysis of 3',5'-disiloxane derivatives

Starting compounds	Products	Yield (%)	^{31}P NMR[a] data	R_f values[b]
10a (R^3 = TIPSi)	**12a** (R^5 = TIPSiOH)	90	−8.06; −8.06	0.59; 0.58
10b (R^3 = TIPSi)	**12b** (R^5 = TIPSiOH)	95	−7.37; −8.07	0.72; 0.70
11 (R^3 = TIPSiOH)	**13** (R^5 = TIPSiOH)	91	−7.38; −7.45	0.65; 0.63
15 (n = 1, R^3 = TIPSi, R^4 = TIPSiOH)	**15** (R^3 = R^5 = TIPSiOH, n = 1)	90	−6.97; −7.02 −7.25; −7.34 −7.49; −7.81	0.68

[a] δ Values are given in ppm; external reference H_3PO_4; solvent $CDCl_3$. [b] Mixture of diastereoisomers (System B).

Table III Relevant data on the synthesis of **14a,b** *and* **15** (n = 1)

Starting compounds	Quantity (mmole)	Base (ml)	Reaction time (min)	Products yield (%)	R_f values[a]
1st component **8** 2nd component **12a**	0.51 0.46	*N*-methyl-imidazole (0.27)	90	**14a** (68)	0.62
1st component **8** 2nd component **12b**	0.24 0.22	Pyridine (0.15)	120	**14b** (56)	0.73
1st component **8** 2nd component **13**	0.18 0.15	*N*-methyl-imidazole (0.08)	120	**15** n = 1 (76.5)	0.67
1st component **8** 2nd component **15** (n = 1) R^3 = R^5 = TIPSiOH	0.16 0.14	*N*-methyl-imidazole (0.07)	120	**15** n = 2 (70)	0.72

[a] System B.

groups from tetramer **15** (n = 2) was effected in the same way as described for the deblocking of trimers **14a,b**. Purification of the crude product by chromatography (Sephadex G25) gave the sodium salt of tetramer **17** as a colourless solid. The identity and homogeneity of **17** thus obtained was unambiguously ascertained by ^{1}H NMR spectroscopy (see Figs. 1-3). The successful synthesis of the three 2–5A core analogues indicates that the bifunctional phosphorylating agent **7** is also applicable to the formation of a 2'–5'-phosphotriester bond between the sterically hindered 2'-OH group of the 3',5'--disilyl-protected riboadenosine **6** and the 5'-OH of another properly protected adenosine unit. Our results also confirm that the acid hydrolysis of the 3',5'-disiloxane bridge to release the 5'-hydroxyl function proceeds with a high degree[4c] of regioselectivity and in good yields (see Table III). In contrast to an earlier report[4c], we found, however, that the presence of the silyl-protecting groups (R^3 and R^5) in the fully protected intermediates **14a,b** and **15** (n = 2) prohibited the removal of the 2-chlorophenyl (R^4) from phosphorus by the well-established oximate-ion-promoted procedure[16]. Thus, prolonged treatment of **14a,b** or **15** (n = 2) with an excess of *syn*-4-nitrobenzaldoxime and N^1,N^1,N^2,N^2-tetramethylguanidine led to incomplete removal of the phosphate-protecting groups (experimental data not given here). Thus, in order to achieve our goal, the simultaneous removal of the base-labile protecting groups (R^4, R^2 and Bz) from **14a,b** as well as from **15** (n = 2) was effected by ammonolysis. In this report it has to be noted that the latter deblocking procedure, in contrast

[15] The synthesis of this compound will be published elsewhere.
[16] *C. B. Reese* and *L. Zard*, Nucleic Acids Res. **9**, 4611 (1981).

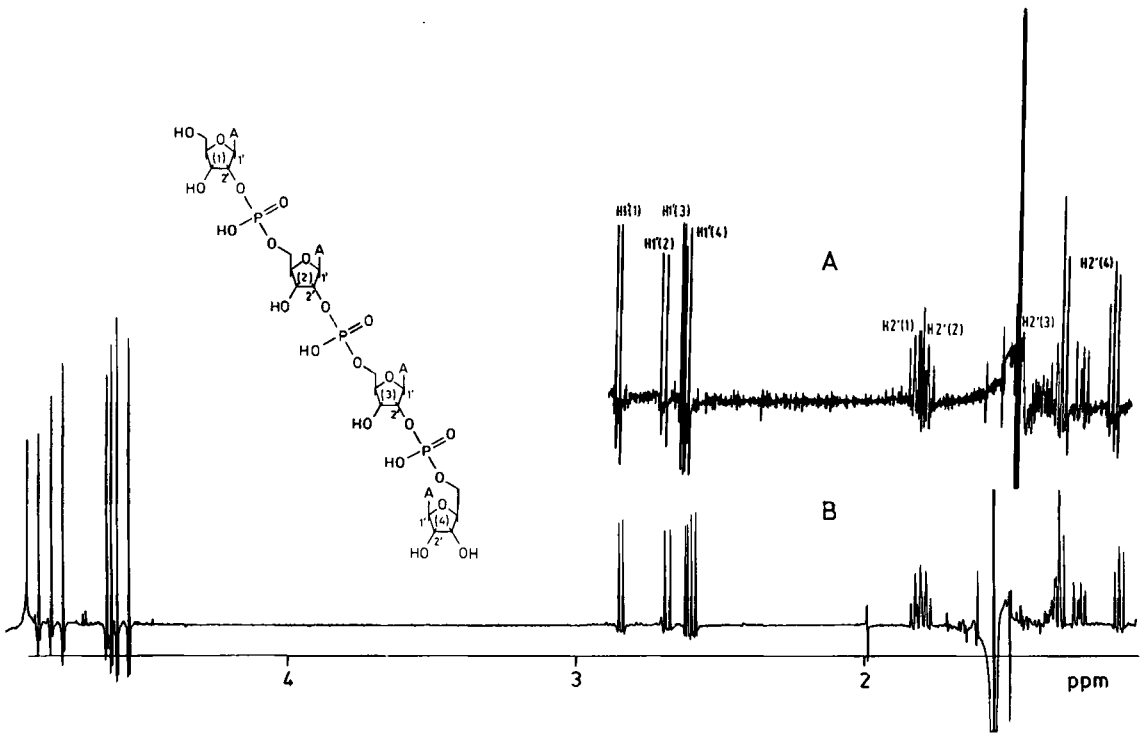

Fig. 1. (A) ^{31}P-coupled ^{1}H NMR spectrum of tetrameter **17**. *(B) ^{31}P-decoupled ^{1}H NMR spectrum of tetramer* **17**. *The correct assignments of the low-field ribose protons H1' and H2' are based upon a ^{31}P-decoupled 2D COSY spectrum.*

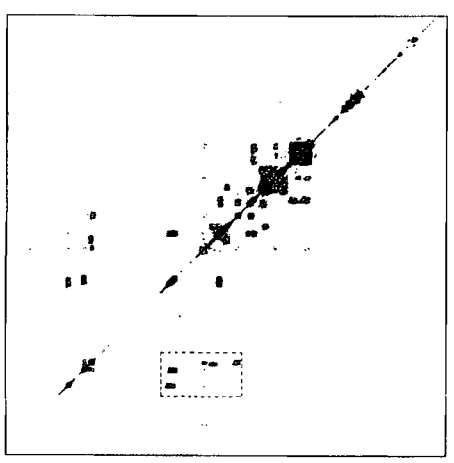

Fig. 2. Contour plot of the ^{31}P-decoupled COSY spectrum (0–3.5 ppm) of tetramer **17** *at 20° C. The shaded area represents the low-field ribose signals.*

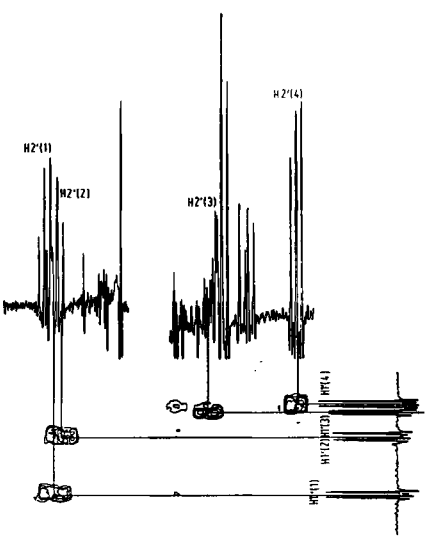

Fig. 3. Expanded contour plot of the ^{31}P-decoupled COSY spectrum of the low-field ribose region. Connectivities between the H1' and H2' ribose protons of residues one to four are indicated by solid lines.

to the oximate-ion process, may result in partial cleavage[17] of the internucleotide linkages. In addition, basic hydrolysis of **15** ($n = 2$) may be accompanied by neighbouring-group participation[18] (NGP) of the liberated terminal 3'-OH group (R^2 = H) on the removal of the 2-chlorophenyl group (R^4) from the terminal 2'–5'-phosphotriester bond. As a result of NGP, tetramer **17** may be contaminated with **17** having a terminal 2'–3'-phosphodiester linkage. Fortunately, however, ^{1}H NMR spectroscopy of purified **17** revealed (see Figs. 1–3) the absence of other than the required 2'–5'-phosphodiester bonds. The 2–5A core analogues **16a,b** have in common the fact that the modifications at the 3'-end of the ribofuranosyl moieties may prohibit cleavage by 2'-PDi, probably without hampering the biological activity[19] of these molecules. Furthermore, the presence of a free 3'-amino group in **16a** may be exploited for the introduction of a suitable fluorescence label. The labelled derivative of **16a** could then serve as a probe in the study of the activation of

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[18a] *J. H. van Boom,. P. M. J. Burgers, P. H. van Deursen, J. F. M. de Rooij* and *C. B. Reese*, J.C.S. Chem. Comm. 167 (1976).
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[19] The biological activity of the 2–5A core analogues is at present under investigation.

RNase L. The presence of the lipophilic group at the 3'-end of **16b** may compensate for the polar character of 2–5A and thereby enhance the penetration through eukaryotic cell membranes. Finally, it may be expected that the L-riboadenosine in tetramer **17** will protect the molecule towards the action of 2'-PDi without affecting the biological activity[16] of the unmodified 2–5A core present in the same molecule.

Experimental

General methods and materials

Dioxane acetonitrile, tetrahydrofuran and pyridine were dried by heating with CaH_2 for 16 h and then distilled. Pyridine used in phosphorylation and condensation reactions was redistilled from *p*-toluenesulphonyl chloride (60 g/l) and KOH (10 g/l). Methanol was dried by refluxing with magnesium methoxide, distilled and stored over molecular sieves 3Å. Toluene and *n*-hexane were dried over sodium wire and all the other solvents over molecular sieves 4Å. 1,2-Dichloroethane was washed with concentrated sulfanic acid, water and 10% aqueous $NaHCO_3$, dried over $CaCl_2$, distilled from P_2O_5 and stored over molecular sieves 4Å. Trimethylsilyl triflate was distilled, carefully sealed and stored at −20°C. *N*-Methylimidazole (Janssen) was dried by heating under reflux with calcium hydride (5 g/l) and distilled under reduced pressure. 1,2--Dichloro-1,1,3,3-tetraisopropyldisiloxane and triethylammonium bicarbonate buffer (2 M) were prepared as previously described[11]. Tetradecanoyl chloride was purchased from Janssen. Short-column chromatography was performed on silica gel (230–240 mesh ASTM). DEAE-Sephadex and Sephadex G-25 were purchased from Pharmacia (Uppsala, Sweden). Schleicher and Schüll DC Fertigfolien F 1500 LS 254 were used for TLC solvent system A (chloroform/methanol, 96/4, v/v, system B (chloroform/methanol, 92/8, v/v) unless otherwise stated. Compounds were visualized by UV light, or by spraying with the appropriate reagents. Thus, compounds containing sugar moieties were visualized by spraying with sulfuric acid (20%, v/v). Compounds containing aliphatic amino groups were detected by ninhydrine spray reagent (Merck). ^{1}H NMR spectra were measured at 100 MHz using a Jeol JNPS 100 spectrometer or at 300 MHz using a Bruker WM 300 spectrometer, equipped with an Aspects 3000 computer, operating in the Fourier Transform mode. ^{13}C NMR spectra were measured at 50.3 MHz using a Jeol JNM-FX 200 spectrometer and ^{31}P NMR spectra were measured at 40.48 MHz using a Jeol PFT 100 spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane (^{1}H NMR) or tetramethylammonium chloride (^{13}C NMR). H_3PO_4 (85%) was used as an external reference for ^{31}P NMR spectroscopy. In spectra measured at 300 MHz (^{1}H NMR), tetramethylammonium chloride (TMA) was used as an internal reference; δ values are given relative to tetramethylsilane (TMS) (δTMA–δTMS = 3.18 ppm). High-performance anion-exchange chromatography was performed using the strong anion-exchange resin Permaphase AAX (Du Pont, USA), dry-packed into a stainless-steel column (1 m × 2.1 mm). Isocratic elution of nucleotides was effected by buffer A (0.05 M KH_2PO_4, pH 4.1) at 20°C with a flow rate of 1 ml/min. Buffer B was composed of 0.05 M KH_2PO_4, 0.5 M KCl, pH 4.5 and buffer C of 0.1 M KH_2PO_4, 1.0 M KCl, pH 4.5. A flow rate of 1 ml/min at a pressure of 8 MP at 20°C was generally used.

1,2-Di-O-acetyl-3-azido-3-deoxy-5-O-benzoyl-β-D-ribofuranose (**1**)

1,2-*O*-Isopropylidene-3-azido-3-deoxy-5-*O*-benzoyl-α-D-ribofuranose (5.11 g, 16.0 mmole) was dissolved in a mixture of acetic acid (120 ml) and acetic anhydride (7.2 ml). To the cooled solution (ice/water bath) was added dropwise (10 min) a mixture of sulfuric acid (3.1 ml) in acetic acid (6.3 ml). After ½ h, the ice/water bath was removed and the solution stirred for a further 15 h at room temperature. The reaction mixture was then diluted with chloroform (200 ml). The organic layer was washed with ice/water (50 ml) and a cold solution of aqueous 5% $NaHCO_3$ (50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$), evaporated to a glass and purified over silica gel (230–240 mesh) suspended in chloroform. Elution of the products with chloroform/methanol (99/1, v/v) afforded, after collection of the appropriate fractions and evaporation of the organic layer, compound **1** as a white glass. Yield 4.43 g (76%). R_f 0.71 (system A). ^{1}H NMR ($CDCl_3$): δ 2.86 (s, 3 × H, 1 × CH_3 acetyl); 2.16 (s, 3 × H, 1 × CH_3 acetyl); 4.30–4.48 (m, 3 × H, H4, H5,5'); 4.60 (d, J_{23} 2.5 Hz, 1 × H, H_3); 5.38 (d, J_{23} 3.5 Hz, 1 × H, H2); 6.15 (s, H1); 7.43–7.57 (m, 3 × H, *p*-, *o*-benzoyl); 7.95–8.11 (m, 2 × H, *m*-benzoyl).

2'-O-Acetyl-3'-azido-3'-deoxy-5'-O-benzoyl-N^6-benzoyladenosine (**3**)

Compound **1** (1.10 g, 3.03 mmole) and silylated compound **2**[8] (2.60 g, 6.79 mmole) were both dissolved in anhydrous 1,2-dichloroethane (55 ml) and coevaporated with anhydrous toluene (2 × 50 ml) to afford an oil. The oil was dissolved in 1,2-dichloroethane (55 ml) and trimethylsilyl triflate (2.6 ml; 1.3 mmole) was quickly added. The mixture was gently refluxed in an atmosphere of dry nitrogen. After 5 h, TLC (system A) showed the reaction to be complete. The reaction mixture was, after cooling down to room temperature, diluted with chloroform (200 ml) and washed with aqueous sodium hydrogen carbonate (10%, v/v, 50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and evaporated to afford a yellow oil. After filtration of unreacted N^6-benzoyladenine, the crude product was dissolved in a mixture of chloroform/methanol (97/3, v/v) and purified on a column (10 × 12 cm^2) of silica gel (230–400 mesh). Elution with the same solvent mixture afforded **3** as a white glass. Yield 1.26 g (77%), R_f 0.67 (system A). ^{1}H NMR ($CDCl_3$): δ 2.16 (s, 3 × H, 1 × CH_3 acetyl); 4.37 (dd, 1 × H, H3'); 4.63 (dd, 2 × H, H5'5''); 4.96 (m, 1 × H, H4'); 6.10 (m, 2 × H, H1', H2'); 7.36–7.51 (m, 3 × H, *p*-, *o*-benzoyl); 7.93–8.11 (m, 2 × H, *m*-benzoyl); 8.18 (s, 1 × H, H2, *exo* cyclic base); 8.53 (s, 1 × H, H8, exocyclic base); 9.44 (s, broad, 1 × H, NH arom.).

3'-Azido-3'-deoxy-N^6-benzoyladenosine (**4**)

Compound **3** (1.33 g, 2.45 mmole) was, after co-evaporation with anhydrous dioxane (2 × 20 ml), dissolved in methanol/dioxane (9.2 ml, 1/1, v/v) and treated with sodium methoxide (1 M, 6.9 ml). After 10 min, the reaction mixture was quenched by adding a slight excess of pyridine HCl salt. The precipitated salts were removed by filtration over Celite Hyflo Supercel and the remaining residue extracted with warm (40°C) methanol/dioxane (60 ml, 1/1, v/v). The filtrate was concentrated under reduced pressure to give a yellow precipitate. The crude product was purified by short-column chromatography. Elution of the column with a mixture of chloroform/methanol (98/2, v/v→90/10, v/v) afforded compound **4**. Yield 0.50 g (52%). R_f 0.44 (system A). ^{1}H NMR ($CDCl_3$): δ 3.72–3.87 (m, 2 × H, H5'5''); 4.12–4.32 (m, 2 × H, H3', H4'); 5.03 (dd, $J_{1'2'}$ 6 Hz, $J_{2'3'}$ 5 Hz, 1 × H, H2'); 6.05 (d, $J_{1'2'}$ 6 Hz, 1 × H, H1'); 7.48–8.57 (m, 3 × H, *p*-, *m*-benzoyl); 8.00–8.07 (m, 2 × H, *o*-benzoyl); 8.55 (s, 1 × H, H2, exocyclic base); 8.65 (s, 1 × H, H8, exocyclic base).

3'-Deoxy-3'-(tetradecanamido)-N^6-benzoyladenosine (**5a**)

Hydrogen gas was bubbled through a solution of compound **4** (0.88 g, 2.22 mmole) in absolute alcohol (70 ml) containing palladium on charcoal (10%, 1.0 g). After 1 h at 20°C, TLC analysis (system B) showed the reaction to be complete. The mixture was filtered over Celite Hyflo Supercel and concentrated to afford 3'-amino-3'--deoxy-*N*^{6}-benzoyladenosine. Yield 0.55 g (67%). R_f 0.20 (system B, ninhydrine positive). The product (0.37 g, 1.00 mmole) was co-evaporated with anhydrous dioxane (3 × 25 ml) and dissolved in anydrous methanol (2.5 ml) and anhydrous dioxane (2.5 ml). After the addition of K_2CO_3 (0.5 g), a mixture of dioxane (3.0 ml) and tetradecanoyl chloride (0.26 g, 1.06 mmole) was added dropwise (10 min) to the solution. TLC analysis (system B), after 1 h, showed the reaction to be complete (R_f 0.20→0.59). After filtration of the salts, the filtrate was diluted with chloroform (200 ml) and washed with aqueous sodium bicarbonate (10%, v/v, 50 ml) and water (50 ml). The organic layer was dried ($MgSO_4$) and evaporated to give a white gelatinous product. Yield 0.42 g, 0.74 mmole (74%). R_f 0.59 (system A). ^{1}H NMR ($CDCl_3$/CD_3OD): δ 0.88 (t, 3 × H, C(O) – CH_2 – CH_2 – $(CH_2)_{10}$ – $\underline{CH_3}$); 1.28 (s, broad, 20 × H, C(O) – CH_2 – CH_2 – $(\underline{CH_2})_{10}$ – CH_3); 1.63 (t, broad, 2 × H, 1 × CH_2, C(O) – CH_2 – $\underline{CH_2}$ – $(CH_2)_{10}$ – CH_3); 2.30 (t, 2 × H, C(O) – $\underline{CH_2}$ – CH_2 – $(CH_2)_{10}$ – CH_3); 3.87 (dd, $J_{5'5''}$ 13 Hz, 1 × H, H5''); 4.03 (dd, $J_{5'5''}$ 13 Hz, 1 × H, H5'); 4.21 (m, 1 × H, H4'); 4.57–4.67 (m, 2 × H, 1 × H2', H3'); 6.16 (d, $J_{1'2'}$ 2 Hz, 1 × H, H1'); 7.57 (t, 2 × H, *m*-benzoyl); 7.66 (t, 1 × H, *p*-benzoyl); 8.11 (d, 2 × H, *o*-benzoyl); 8.76 (s, 2 × H, H2 and H8, exocyclic base). ^{13}C NMR ($CDCl_3$/CD_3OD): δ 14.1 (s, C(O) – $(CH_2)_{12}$ – $\underline{CH_3}$); 23.1, 26.1, 29.9, 32.2, 36.5 (s, C(O) – $(\underline{CH_2})_{12}$ – CH_3); 61.5 (s, C5'); 74.7 (s, C2'); 84.6 (s, C4'); 91.6 (s, C1'); 128.9, 130.1, 132.2 (s, benzoyl); 128.5 (s, C5); 142.6 (s, C8); 149.7 (s, C4); 150.8 (s, C2); 152.4 (s, C6).

3',5'-O-(Tetraisopropyldisiloxane-1,3-diyl)-N^6-benzoyladenosine (**6**)

N^6-Benzoyladenosine (3.7 g, 10 mmole) was dissolved in anhydrous pyridine (52 ml) and to the magnetically stirred solution was added a solution of 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane (7.2 ml, 15 mmole) in pyridine (10 ml). TLC analysis (system A), after 1 h,

showed the reaction to be complete. The reaction was quenched by the addition of triethylammonium bicarbonate (1 M, TEAB; 30 ml, pH 7.5) and the mixture was dissolved in chloroform (200 ml) and washed with water (2 × 50 ml). The organic layer was dried ($MgSO_4$)) and evaporated to dryness. The crude product was dissolved in chloroform and applied to a column of silica gel (230–400 mesh) suspended in chloroform. The column was eluted with chloroform and the appropriate fractions were collected and concentrated to an oil. Compound **6** was crystallized from *n*-hexane. Yield 4.31 g (70%) as white crystals. R_f 0.66 (system A). M.p. 83–85°C. ^{1}H NMR ($CDCl_3$): δ 1.15 (m, 24 × H, 4 × *i*Pr); 4.12 (m, 3 × H, H4′, H5′5″); 4.42 (d, 1 × H, H3′); 5.03 (m, 1 × H, H2′); 6.0 (s, 1 × H, H1′); 7.30–8.13 (m, 5 × H, benzoyl); 8.21 (s, 1 × H, H2, exocyclic base); 8.72 (s, 1 × H, H8, exocyclic base). ^{13}C NMR ($CDCl_3$): δ 12.3, 12.6, 12.8, 13.3 (s, 4 × Si-C); 17.1, 18.0 (s, 8 × CH_3 TIPDSi); 61.4 (s, C5); 70.5 (s, C2′); 75.0 (s, C3′); 82.2 (s, C5′); 90.0 (s, C1′); 128.1, 128.7, 132.7, 133.5 (s, benzoyl); 123.3 (s, C5); 141.9 (s, C8); 149.9 (s, C4); 150.8 (s, C6); 152.6 (s, C2); 165.2 [s, 1 × C(0)].

General procedure for the preparation of the fully protected dimers **10a,b**, **11**, *trimers* **14a,b** *and tetramer* **15** *(n = 2)*
Synthesis of the fully protected dimers **10a,b** *and* **11**

Preparation of intermediate **8** *(R^3 = TIPDSi)**. A solution of 2-chlorophenyl*bis*(1-benzotriazolyl) phosphate **7** in anhydrous dioxane (1.15 eq from a 0.2 M stock solution) and 1-methylimidazole (5 eq.) were added, under anhydrous conditions, to partially protected nucleoside **6** (1.0 mmole). After stirring for 30 min at 20°C, TLC analysis (system A) showed compound **6** to be completely converted into **8** (R_f 0.0). The solution of intermediate **8** thus obtained was immediately used for the preparation of compounds **10a,b**, **11**, **14a,b** and **15** (*n* = 1, 2).
Preparation of dimers **10a,b** *and* **11**. To the solution of intermediate **8** (R^3 = TIPDSi, R^4 = 2-chlorophenyl, 1.0 mmole) in dioxane (5.75 ml) was added one of the modified building blocks **5a** (0.8 eq); **5b** (1 eq.) or **9** (0.9 eq.). The synthesis of dimer **10b** was performed in anhydrous tetrahydrofuran. TLC analysis (system A), after 1½ h at 20°C, showed the reaction to be complete. The reaction mixture was diluted with chloroform (75 ml) and washed with triethylammonium bicarbonate (IM, 30 ml). The organic layer was dried ($MgSO_4$) and concentrated to an oil. Crude compounds **10a,b** and **11** were purified by short-column chromatography silica gel (230–400 mesh). Evaporation of the appropriate fractions afforded the fully protected dimers **10a,b** and **11** in good yields. Data on the coupling conditions and yields of dimers **10a,b** and **11** are summarized in Table I.

General procedure for the selective hydrolysis of 3′,5′-TIPDSi-protected oligomers **10a,b**, **11** *and* **15** *(n = 1) to afford the acyclic 3-hydroxy-1,1,3,3-tetraisopropyldisiloxanyl (TIPSiOH) derivatives* **12a,b**, **13** *and* **15** *(n = 1, $R^3 \rightarrow R^5$).*

The 3′,5′-*O*-TIPDSiprotected dimers **10a,b**, **11** (1.0 mmole) or trimer **15** (*n* = 1; 1.0 mmole) were dissolved in freshly distilled dioxane (126 ml). To the magnetically stirred solutions was added dioxane (23 ml) and aqueous HCl (12 N, 2.1 m). After stirring for 1 h at 20°C, TLC analysis (system A) showed conversion of the starting products into slower-running compounds. The solution was neutralized with sodium bicarbonate (5%) and extracted twice with chloroform. The organic layer was dried ($MgSO_4$) and evaporated to a white glass. Short-column purification afforded the 3′-TIPSiOH products in good yield. Data on the yields of the pure dimers **12a,b**, **13** and trimer **15** (*n* = 1; $R^3 \rightarrow R^5$) are summarized in Table II.

Synthesis of the fully protected trinucleotides **14a,b** *and tetranucleotide* **15** *(n = 2)*

The 3′-TIPSiOH nucleotides **12a,b** and **13** were, after co-evaporation twice with anhydrous pyridine (2 × 10 ml), condensed with compound **8**, as described for the synthesis of dimers **10a,b** and **11**. In the same way, tetramer **15** (*n* = 2) was obtained. Data on the yields of the pure trinucleotides **14a,b** and tetranucleotide **15** (*n* = 2) are summarized in Table III.

General procedure for deblocking of oligonucleotides **14a,b** *and* **15** *(n = 2)*

To a solution of one of the fully protected compounds **14a,b** or **15** (*n* = 2) (0.05 mmole) in dioxane (10 ml) was added aqueous ammonia (25%, 10 ml). The reaction vessel was sealed and maintained at 50°C for a period of 5 h (oil-bath) after which time the mixture was evaporated to give a white residue. After co-evaporation with anhydrous pyridine (1 × 15 ml), the residue was dissolved in pyridine (8 ml) and a solution of tetrabutylammonium fluoride in anhydrous tetrahydrofuran (0.2 M TBAF, 4 eq.) was added. The mixture was magnetically stirred for 16 h at 20°C and then diluted with water (8 ml). The filtrate was evaporated to a small volume and extracted with chloroform (2 × 15 ml). The crude product **16a** was purified on a DEAE-Sephadex A-25 column (HCO_3^- form) suspended in 0.05 M triethylammonium bicarbonate. The column was eluted with a linear gradient of 0.05 → 1.0 M triethylammonium bicarbonate for 16 h at a flow-rate of 40 ml/h. The appropriate fractions (volume 8 ml) were pooled and lyophilized. Compound **17** was purified by passing it through a column (2 m × 3 cm^2) of Sephadex G-25 suspended in 0.05 M triethylammonium bicarbonate. Elution was effected with the same buffer at a flow-rate of 40 ml/h. Fractions of 5 ml were taken and analyzed by HPLC (AAX column). The fractions containing the pure product were collected and lyophilized. The products **15a,b** and **17** were brought into the sodium form by passing them over a column (15 × 2 cm^2) of Dowex 50W cation-exchange resin (100–250 mesh, sodium form) and the resulting aqueous solutions were lyophilized.
Compound **14a** (0.05 mmole) was deblocked as described above. The TBAF treatment was performed in anhydrous pyridine (5 ml). Yield of **16a** (Na^+ form) after work-up and purification (DEAE-Sephadex A-25): 0.031 g (61%). ^{1}H NMR (300 MHz) (D_2O): δ 3.68 (dd, 2 × H, H5′5″, J_{gem} 13.5 Hz free end); 4.75 (m, 2 × H, 2 × H2′); 5.64 (d, 1 × H, $J_{1'2'}$ 2.0 Hz, H1′); 5.77 (d, 1 × H, $J_{1'2'}$ 4.5 Hz, H1′); 5.87 (d, 1 × H $J_{1'2'}$ 4.0 Hz, H1′); 7.57, 7.69, 7.73, 7.84, 7.89, 8.01 (6 × s, 6 × H, 3 × H8, 3 × H2, exocyclic base).
Compound **14b** (0.43 mmole) was deblocked as described above except that the TBAF treatment was performed in a mixture of pyridine/THF (10 ml, 6/4, v/v). Yield of **16b** (Na^+ form) after work-up and purification (DEAE-Sephadex A-25): 0.028 g (58%). ^{1}H NMR (300 MHz) (D_2O): 0.53 (3 × H, 1 × CH_3, C(O) – CH_2 – – CH_2 – $(CH_2)_{10}$ – $\underline{CH_3}$); 0.90 (20 × H, C(O) – CH_2 – CH_2 – – $(\underline{CH_2})_{10}$ – CH_3); 1.38 (2 × H, C(O) – CH_2 – $\underline{CH_2}$ – $(CH_2)_{10}$ – – CH_3); 2.12 (2 × H, C(O) – $\underline{CH_2}$ – $(CH_2)_{10}$ – CH_3); 3.50 (dd, 2 × H, H5′5″, free end); 4.83 (m, 2 × H, 2 × H2′); 5.67 (s, 1 × H, H1′); 5.80 (s, 1 × H, H1′); 5.87 (s, 1 × H, H1′); 7.65, 7.68, 7.82, 7.87, 7.92, 7.97 (6 × s, 6 × H, 3 × H8, 3 × H2, exocyclic base).

Deblocking of compound **15** *(n = 2)*

Compound **15** (*n* = 2; 0.041 mmole) was deblocked as described above except that the TBAF treatment was performed in anhydrous THF (8 ml). Yield of **17** (Na^+ form) after work-up and purification (DEAE-Sephadex G-25): 0.032 g (6.34%). ^{1}H NMR (300 MHz) (D_2O): δ 3.65 (dd, 2 × H, H5′5″, free end); 4.53 [m, 1 × H, H2′(3)]*; 4.28 [t, 1 × H, H2′(4)]; 4.95 [m, 2 × H, 2 × H2′, H2′(1), H2(3)]; 5.75 [d, 1 × H, $J_{1'2'}$ 4.0 Hz, H1′, (C4)]; 5.78 [d, 1 × H, $J_{1'2'}$ 2.0 Hz, H1′(3)]; 5.87 [d, 1 × H, $J_{1'2'}$ 5.1 Hz, H1′(2)]; 6.02 [d, 1 × H, $J_{1'2'}$ 4.0 Hz, H1′(1)]; 6.70, 6.77, 6.78 (s, 4 × H, 4 × H2, exocyclic base); 6.92, 6.97, 7.30, 7.12 (s, 4 × H, 4 × H8, exocyclic base).

* TIPDSi = 1,1,3,3-tetraisopropyldisiloxane-1,3-diyl.

* Number in parenthesis refers to nucleoside unit: for details see Fig. 1.